

## Adenylate cyclase and acetylcholine release regulated by separate serotonin receptors of somatic cell hybrids

(lysergic acid diethylamide/neurotransmitters/synapse/neuroblastoma)

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**ABSTRACT** Serotonin activates adenylate cyclase [ATP pyrophosphate-lyase (cyclizing), EC 4.6.1.1] of NCB-20 neuroblastoma-brain hybrid cells with an activation constant of 530 nM, but has little or no effect on cellular cyclic AMP or cyclic GMP content of N1E-115 neuroblastoma or NG108-15 hybrid cells. In homogenates of NCB-20 hybrid cells, lysergic acid diethylamide stimulates adenylate cyclase activity ( $K_{act} = 12$  nM) and partially inhibits ( $K_i = 10$  nM) the stimulation of adenylate cyclase activity by serotonin. No desensitization was detected of serotonin receptors coupled to adenylate cyclase. Serotonin also depolarizes NCB-20, NG108-15, and N1E-115 cells and increases acetylcholine release. Serotonin receptors mediating depolarizing responses desensitize rapidly and reversibly, and the depolarizing effects of serotonin are neither mimicked nor inhibited by lysergic acid diethylamide. These results indicate that (i) NCB-20 cells possess at least two species of serotonin receptors, which independently regulate cellular functions, (ii) activation of adenylate cyclase does not directly affect membrane potential or acetylcholine release, and (iii) serotonin-dependent cell depolarization does not affect cyclic AMP or cyclic GMP synthesis in the cell lines tested.

Serotonin activates adenylate cyclase [ATP pyrophosphate-lyase (cyclizing), EC 4.6.1.1] in mammalian brain (1-3) and increases or decreases the frequency of spontaneous action potentials in cerebral neurons (4, 5). Serotonin also induces contractions in ileal smooth muscle (6). However, central neuronal responses to serotonin in mammals are predominantly inhibitory and are mediated through either pre- or postsynaptic receptors, which can be distinguished by the differences in their responses to D-lysergic acid diethylamide (LSD) (5, 7). In molluscan neurons, as many as six kinds of response to serotonin have been detected (8).

Serotonin-dependent activation of adenylate cyclase is preserved after electrolytic lesion of the raphe nuclei, which greatly reduces the number of serotonergic neurons innervating the colliculus. This suggests that collicular serotonin receptors that mediate activation of adenylate cyclase are postsynaptic receptors (2).

In this report, effects mediated by two species of serotonin receptor in NCB-20 neuroblastoma-brain hybrid cells are described. One receptor is coupled to the activation of adenylate cyclase, and the other mediates cell depolarization and acetylcholine release. The receptors are distinguished on the basis of functional response, specificity, and desensitization.

## MATERIALS AND METHODS

**Cells.** The N1E-115 adrenergic neuroblastoma cell line was cloned from C-1300 mouse neuroblastoma (9). The NG108-15 neuroblastoma-glioma hybrid cell line (unpublished results) was derived by Sendai virus-induced fusion of C-1300 mouse neuroblastoma clone N18TG2 resistant to 6-thioguanine (10) with rat glioma clone C6BU-1, resistant to 5-bromodeoxyuridine (11). The NCB-20 neuroblastoma-fetal Chinese hamster brain hybrid cell line (12) was obtained by Sendai virus-induced fusion of N18TG2 with fetal Chinese hamster brain cells dissociated from 18-day embryos. The NBr-10A and NBr-20A neuroblastoma-rat liver hybrid cell lines (unpublished results) were obtained by Sendai virus-induced fusion of N18TG2 with the BRL30E Buffalo rat liver cell line, resistant to 5-bromodeoxyuridine (H. G. Coon, personal communication).

**Adenylate Cyclase Assay.** Cells were cultured as described (13). Confluent cells were detached from flasks by gentle tapping and were washed three times, each with 10 ml of Dulbecco's phosphate-buffered saline (without  $Ca^{2+}$  or  $Mg^{2+}$  ions) adjusted to 340 mOsm with NaCl. Cells were recovered by centrifugation ( $150 \times g$  for 5 min) after each wash, and after the third wash were suspended in 25 mM Tris-HCl, pH 7.4/290 mM sucrose (2 ml/75-cm<sup>2</sup> flask) and homogenized at 4°C with 50 strokes of a Dounce homogenizer. Homogenates were frozen and stored in a liquid N<sub>2</sub> freezer. Each 100- $\mu$ l reaction mixture contained 50 mM Tris-HCl (pH 7.4); 87 mM sucrose; 20 mM creatine phosphate, disodium salt (Sigma); 10 International Units of creatine kinase, 150 units/mg of protein (ATP:creatine N-phosphotransferase, EC 2.7.3.2) from Sigma; 1 mM cyclic AMP (cAMP), sodium salt (Sigma); 0.25 mM Ro20-1724 (a gift from Hoffmann-La Roche); 0.25% ethanol; 1 mM [ $\alpha$ -<sup>32</sup>P]ATP (3  $\mu$ Ci, New England Nuclear; 1 Ci =  $3.7 \times 10^{10}$  Bq); and 100-200  $\mu$ g of homogenate protein.

Homogenates were thawed and maintained at 4°C in an ice bath for no longer than 10 min prior to incubation. Reaction mixtures were incubated for 8 min at 37°C. Adenylate cyclase activity was determined by a modification (14) of method C

Abbreviations: LSD, D-lysergic acid diethylamide; PGE<sub>1</sub>, prostaglandin E<sub>1</sub>; cAMP, cyclic AMP; Bt<sub>2</sub>cAMP, N<sup>6</sup>, O<sup>2'</sup>-dibutyryl cyclic AMP; cGMP, cyclic GMP; Gpp(NH)p, guanylyl-5'-imidodiphosphate.

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of Salomon *et al.* (15). The production of [ $^{32}$ P]cAMP was proportional to protein concentration within the range 50–250  $\mu$ g of homogenate protein per reaction mixture; similarly [ $^{32}$ P]-cAMP synthesis increased linearly for 30 min. Values are the means of two to four determinations, except where noted; replicates usually varied by <8%.

**cAMP and Cyclic GMP (cGMP) Assays.** Methods for cell culture and incubation, and for cAMP and cGMP separation and assay have been described by Matsuzawa and Nirenberg (16). Protein was determined by a modification of the method of Lowry *et al.* (17), with bovine serum albumin, crystallized three times, as standard.

**Electrophysiological Measurements.** Hybrid cells were usually cultured for 5–15 days in the presence of 1 mM  $N^6$ ,  $O^2$ -dibutyryl-cAMP (Bt<sub>2</sub>cAMP). Myoblasts were dissociated from newborn Fisher rat hind limb and cultured for 3 days. Nondividing myotubes were selected for by incubating cells with 10  $\mu$ M 5-fluorodeoxyuridine and 100  $\mu$ M uridine for 48 hr; then hybrid cells that had been treated with 1 mM Bt<sub>2</sub>cAMP for 7–10 days were added and cells were cocultured for an additional 1–3 days in the presence of 1 mM Bt<sub>2</sub>cAMP (18). The techniques for intracellular recording of membrane potentials of cultured cells have been described (18, 19). Serotonin and LSD were applied to cells iontophoretically or by addition of a solution from a micropipette.

The following generous gifts were received: LSD from Judith R. Walters; bufotenine-creatinine sulfate (Upjohn) and  $N,N'$ -dimethyl-5-methoxytryptamine (free base) (Aldrich) from J. C. Gillin; mianserin-hydrochloride (Organon Products, West Orange, NJ) from S. H. Snyder; methysergide-hydrogen maleate from C. R. Creveling; prostaglandin E<sub>1</sub> (PGE<sub>1</sub>) from Upjohn; metergoline from Farmitalia (Milan, Italy); cyproheptadine-hydrochloride from Merck, Sharp and Dohme; fluphenazine-2 HCl from Squibb; phentolamine-hydrochloride from Ciba Pharmaceuticals (Summit, NJ); and pimozide (free base) from McNeil Laboratories Inc. (Fort Washington, PA). Serotonin-creatinine sulfate, 5-methoxytryptamine-hydrochloride, and propranolol-hydrochloride were obtained from Sigma; tryptamine-hydrochloride from Regis Chemical Co.; and guanylyl-5'-imidodiphosphate [GPP(NH)p] from P-L Biochemicals.

## RESULTS

Serotonin stimulated adenylate cyclase activity 75–115% in homogenates of NCB-20 hybrid cells (Fig. 1). The serotonin concentration required for half-maximal activation ( $K_{act}$ ) was consistently within the range 400–800 nM and was not affected by 50  $\mu$ M ascorbate or 100  $\mu$ M pargyline. Homogenates were prepared and frozen for subsequent use since freezing and thawing had little or no effect on the serotonin-dependent stimulation of adenylate cyclase. An Eadie-Scatchard plot (Fig. 1, upper inset) was linear and the  $K_{act}$  for serotonin was calculated to be 530 nM. From a Hill plot (Fig. 1, lower inset), the interaction coefficient ( $n$ ) was found to be 1.0, revealing no cooperativity in the serotonin-dependent activation of adenylate cyclase. [Ethylenebis(oxyethylenenitrilo)]tetraacetic acid (EGTA; 1–10,000  $\mu$ M) had no effect on the stimulation of adenylate cyclase activity by serotonin, which suggests the effect of serotonin is not dependent on  $Ca^{2+}$  ions (data not shown).

Adenylate cyclase also was stimulated by LSD ( $K_{act}$  = 12 nM) and the LSD analogs metergoline ( $K_{act}$  = 250 nM) or methysergide ( $K_{act}$  = 620 nM) (Fig. 2A). The maximum stimulations of adenylate cyclase activity by LSD or LSD analogs were less than those obtained in the presence of serotonin (Fig. 1). The synthesis of [ $^{32}$ P]cAMP in the presence or absence of 10  $\mu$ M

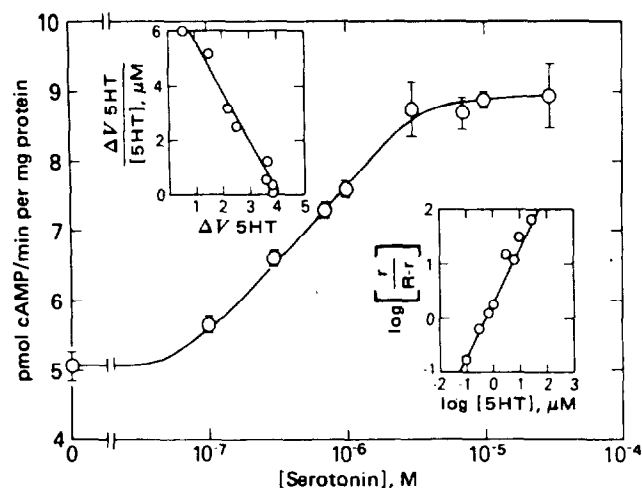


FIG. 1. Serotonin-dependent increase in adenylate cyclase activity in a homogenate of NCB-20 hybrid cells. Results show the means ( $\pm$ SD) of triplicate determinations of adenylate cyclase activity as a function of serotonin concentration. (Upper Inset) Eadie-Scatchard plot;  $\Delta V$  5HT is the serotonin-dependent increase in [ $^{32}$ P]cAMP synthesis (pmol/min per mg of protein). (Lower Inset) Hill plot; "r" is the increase in adenylate cyclase activity at each serotonin concentration and "R" is the maximum increase.

serotonin or 0.1  $\mu$ M LSD was linear with respect to time and did not desensitize within 16 min (Fig. 2B). In another experiment, no desensitization to serotonin was detected in 30 min (not shown).

Adenylate cyclase activity also was increased by the following analogs of serotonin: tryptamine, bufotenine, 5-methoxytryptamine, and  $N,N'$ -dimethyl-5-methoxytryptamine (Fig. 3A). The  $K_{act}$  values for these compounds were 400–570 nM, and maximum stimulations of adenylate cyclase activity were similar to that of serotonin (Table 1).

The serotonin receptor antagonists, mianserin or cyproheptadine, inhibited the stimulation of adenylate cyclase by 10  $\mu$ M serotonin (Fig. 3B). The  $K_i$  values were 43 nM for mianserin and 95 nM for cyproheptadine. Mianserin and cyproheptadine also inhibited the stimulation of adenylate cyclase by LSD ( $K_i$  = 100 nM and 64 nM, respectively). The dopamine receptor antagonists, fluphenazine or pimozide, inhibited serotonin-dependent stimulation of adenylate cyclase ( $K_i$  = 47 nM and

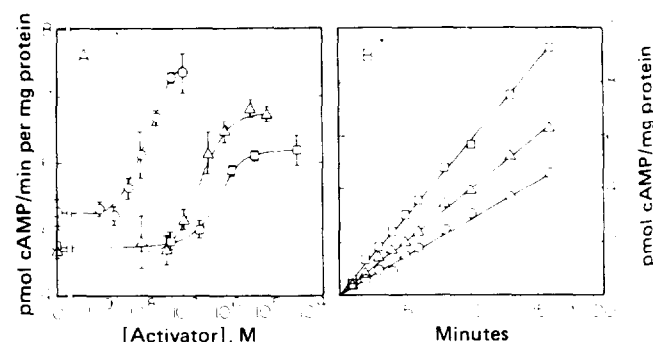


FIG. 2. Activation of adenylate cyclase in homogenates of NCB-20 hybrid cells as a function of concentration of lysergic acid derivatives (A) or by LSD and serotonin as a function of incubation time (B). (A) Values are the means ( $\pm$ SD) of triplicate determinations:  $\circ$ , LSD;  $\Delta$ , metergoline; and  $\square$ , methysergide. (B) Volume of each reaction mixture was 2 ml; concentrations of reagents are given in Materials and Methods. At each time indicated, a 100- $\mu$ l aliquot was removed and the reaction was terminated. Adenylate cyclase activity was determined in the presence of water ( $\circ$ ); 0.1  $\mu$ M LSD ( $\Delta$ ); 10  $\mu$ M serotonin ( $\square$ ).

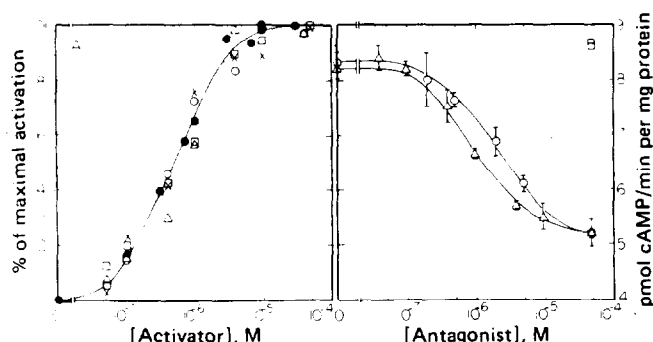


FIG. 3. Activation of adenylate cyclase by tryptamine derivatives (A) and the inhibition of serotonin-stimulated adenylate cyclase activity in homogenates of NCB-20 hybrid cells by mianserin and cyproheptadine (B). (A) Values are the means of triplicate determinations of adenylate cyclase activity in the presence of increasing concentrations of tryptamine derivatives: ●, serotonin [3.89]; ○, tryptamine [3.90]; Δ, bufotenine [2.75]; ○, 5-methoxytryptamine [3.98]; or x, *N,N'*-dimethyl-5-methoxytryptamine [3.51]. (Values shown in brackets are the pmol of cAMP/min per mg of protein that correspond to 100% on the ordinate.) (B) Results show the means (±SD) of triplicate determinations of adenylate cyclase activity in the presence of 10 μM serotonin and increasing concentrations of either mianserin (Δ) or cyproheptadine (○). Basal enzyme activity was 4.93 (±0.27) pmol of cAMP/min per mg of protein.

250 nM, respectively). These  $K_i$  values are greater than those reported for the inhibition of [<sup>3</sup>H]haloperidol binding to dopamine receptors in calf brain membranes (0.88 nM and 0.81 nM, respectively) (20). The α- or β-adrenergic receptor antagonists, phentolamine or propranolol, respectively, had little or no effect on the stimulation of adenylate cyclase by serotonin. Results are summarized in Table 2.

The effect of serotonin concentration on adenylate cyclase activity in the presence or absence of a saturating concentration of LSD (10 μM) is shown in Fig. 4A. LSD stimulated adenylate cyclase and also partially inhibited the serotonin-dependent increase in enzyme activity. The effect of LSD concentration on adenylate cyclase activity was determined by using a different homogenate in the presence or absence of 10 μM serotonin (Fig. 4B). LSD increased adenylate cyclase activity ( $K_{act}$  = 12 nM), as shown previously, and also partially inhibited the serotonin-dependent activation of adenylate cyclase ( $K_i$  = 10 nM).

Serotonin stimulated adenylate cyclase activity only slightly

Table 1. Specificity of serotonin receptors coupled to adenylate cyclase of NCB-20 hybrid cells

Additions	$K_{act}$ , nM	Maximum activation, %
Tryptamine derivatives		
Serotonin	530	80
5-Methoxytryptamine	570	78
Tryptamine	400	77
<i>N,N'</i> -Dimethyl-5-methoxytryptamine	410	73
Bufotenine	520	60
Lysergic acid derivatives		
LSD	12	41
Metergoline	250	45
Methysergide	620	32

The activation constant ( $K_{act}$ ) for each compound is the concentration required for half-maximal stimulation of adenylate cyclase activity. The salts of the compounds tested are given in *Materials and Methods*.

Table 2. Effects of receptor antagonists on serotonin- or LSD-activated adenylate cyclase of NCB-20 hybrid cells

Additions	Adenylate cyclase, $K_i$ (nM)	
	5HT-activated	LSD-activated
Mianserin	43	100
Cyproheptadine	95	64
LSD (partial agonist)	10	
Fluphenazine	47	
Pimozide	250	
Phentolamine	>10,000	
Propranolol	>10,000	

The  $K_i$  values were calculated from the following equation:  $K_i = IC_{50}/1 + ([activator]/K_{act} \text{ of activator})$ , where  $IC_{50}$  is the concentration of antagonist producing half-maximal inhibition. 5HT, serotonin (5-hydroxytryptamine).

in homogenates prepared from NG108-15, NBr-10A, NBr-20A hybrid, or parental N18TG2 neuroblastoma cells (Table 3). Treatment of intact NG108-15 or N1E-115 cells with 10 μM serotonin for 0.5, 1.0, 1.5, 2.0, 2.5, 5.0, and 10 min had little or no effect on intracellular levels of cAMP or cGMP (not shown).

As shown in Table 4, GTP increased basal adenylate cyclase activity by 31% and increased serotonin-dependent activation by 265%. Sodium fluoride stimulated adenylate cyclase activity 850% and completely blocked the effect of serotonin. Gpp(NH)p also stimulated adenylate cyclase activity 125%; however, the stimulatory effects of Gpp(NH)p and serotonin were additive. PGE<sub>1</sub> activated adenylate cyclase, and serotonin reduced by 13% the PGE<sub>1</sub>-dependent activation of the enzyme. Sodium fluoride or Gpp(NH)p inhibited PGE<sub>1</sub>-stimulated adenylate cyclase activity, and serotonin further reduced the activity in the presence of PGE<sub>1</sub> and Gpp(NH)p. These results suggest that activation of adenylate cyclase by serotonin requires GTP and reveals no unusual interaction with sodium fluoride or PGE<sub>1</sub>, with respect to the coupling of serotonin receptors to the adenylate cyclase complex. However, the additive rather than synergistic effects of serotonin and Gpp(NH)<sub>2</sub> may indicate a difference in the mechanism of coupling of serotonin receptors to adenylate cyclase compared to other species of receptors.

Responses of a NG108-15 hybrid cell to serotonin applied iontophoretically are presented in Fig. 5, which are representative of the responses of NCB-20 and N1E-115 cells. Serotonin application resulted in two action potentials followed by cell depolarization for more than 1 sec (Fig. 5A), confirming previous findings (19). Repetitive application of serotonin, at a

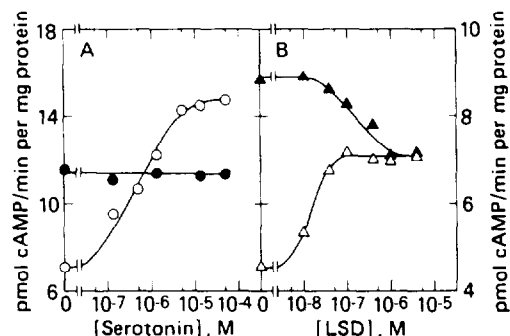


FIG. 4. Effect of LSD and serotonin on adenylate cyclase activity in homogenates of NCB-20 hybrid cells. (A) Increase in adenylate cyclase activity in response to increasing concentrations of serotonin in the absence (○) or presence (●) of 10 μM LSD. (B) Response to increasing concentrations of LSD in the absence (Δ) or presence (▲) of 10 μM serotonin.

Table 3. Effect of serotonin (5HT) on adenylate cyclase activity in homogenates of neuroblastoma N18TG2 or hybrid cell lines

Cell line	pmol cAMP/min per mg protein		Activation, %
	Basal	10 $\mu$ M 5HT	
NCB-20	7.03	14.8	111
NBr-20A	7.08	8.36	18
NG108-15	14.2	16.5	16
N18TG2	4.09	4.24	4
NBr-10A	48.7	50.0	3

frequency of 0.51 Hz, almost completely desensitized the response to serotonin (Fig. 5B). Responsiveness to serotonin recovered within a few minutes in the absence of serotonin. LSD did not inhibit serotonin-dependent cell depolarization (Fig. 5C), and furthermore addition of LSD did not depolarize the cell (not shown). Application of PGE<sub>1</sub>, which markedly increases adenylate cyclase activity (Table 4), had little or no effect on cell membrane potential (unpublished results) or acetylcholine release (21) from NG108-15 cells.

NG108-15 (18) and NCB-20 (unpublished results) hybrid cells form synapses with rat striated muscle cells *in vitro*. Serotonin-dependent release of acetylcholine from an NG108-15 hybrid cell at a synapse was measured by intracellular recording of synaptic potentials in a rat myotube (Fig. 6A). Application of serotonin to the hybrid cell resulted in an increase in acetylcholine secretion from the NG108-15 hybrid cell and a greater than 50-fold increase in the frequency of muscle synaptic responses. LSD neither mimicked nor inhibited serotonin-dependent synaptic responses of the muscle cell (not shown). Serotonin had no effect on the membrane potential of muscle cells that were not innervated by NG108-15 or NCB-20 cells.

The effect of serotonin on [<sup>3</sup>H]acetylcholine release from NG108-15 cells into the medium was measured. Muscle cells were not present. Cells were incubated with [<sup>3</sup>H]choline and washed and [<sup>3</sup>H]acetylcholine released into the medium was separated from [<sup>3</sup>H]choline and assayed (Fig. 6B) (21). Serotonin stimulated acetylcholine release from cells as shown (21), and acetylcholine release was not inhibited by 50–500 nM LSD. The same concentrations of LSD had little or no stimulatory effect on acetylcholine release.

Table 4. Effect of GTP, sodium fluoride, Gpp(NH)p, PGE<sub>1</sub>, and serotonin on NCB-20 adenylate cyclase activity

Addition	pmol cAMP/min per mg protein	
	Basal	10 $\mu$ M 5HT
Exp. A		
Control	16.3	20.0
1 $\mu$ M GTP	21.4	31.2
Exp. B		
Control	5.06	8.88
20 mM NaF	43.5	42.6
10 $\mu$ M Gpp(NH)p	11.4	16.6
10 $\mu$ M PGE <sub>1</sub>	90.2	78.2
10 $\mu$ M PGE <sub>1</sub> + 20 mM NaF	58.1	55.0
10 $\mu$ M PGE <sub>1</sub> + 10 $\mu$ M Gpp(NH)p	86.4	75.7

In Exp. A, each reaction mixture contained 81.6  $\mu$ g of protein of a 30,000  $\times$  g particulate fraction, washed three times with 25 mM Tris-HCl (pH 7.4), recovered by centrifugation (20 min). In Exp. B, adenylate cyclase activity of an unfractionated NCB-20 homogenate is shown. Each value is the mean of triplicate determinations. 5HT, serotonin.

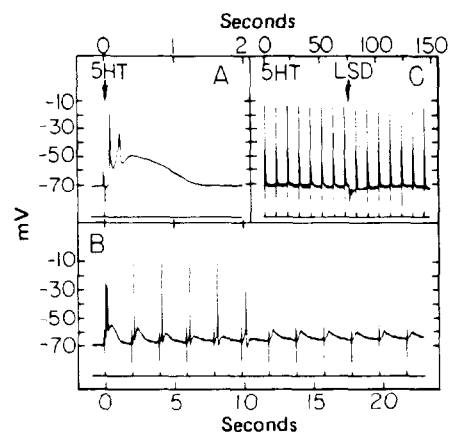


FIG. 5. Serotonin (5HT)-dependent regulation of ionophore activity in NG108-15 hybrid cells. (A) Iontophoretic application of serotonin to a hybrid cell. The iontophoretic pipette contained a solution of 25 mM serotonin in water; a current of 100 nA was passed for 1 msec (shown in the trace at the bottom of each panel). (B) Repetitive serotonin application at a frequency of 0.51 Hz, which results in desensitization. (C) Effect of LSD on the depolarizing response was determined with repeated iontophoretic applications of serotonin in the absence of LSD and then after addition of 20  $\mu$ l of 100  $\mu$ M LSD in 150 mM NaCl. Prior to each pulse, a voltage calibration of 10 mV was passed for 10 msec.

## DISCUSSION

Activation of serotonin receptors of NG108-15 (19, 21) or NCB-20 hybrid cells results in cell depolarization, action potentials, and release of acetylcholine into the medium. These responses desensitize in less than 15 sec and are not inhibited

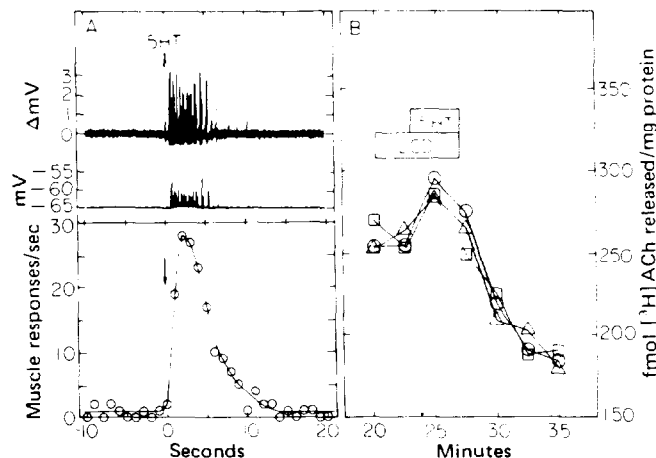


FIG. 6. Serotonin-dependent release of acetylcholine from NG108-15 hybrid cells. (A) Depolarizing responses of a rat striated muscle cell to acetylcholine released from a NG108-15 cell in response to serotonin (5HT) at a synapse; recording obtained with an intracellular microelectrode. Two microliters of a solution containing 10  $\mu$ M serotonin and 150 mM NaCl was applied to the hybrid cell (arrow). (Top) Trace of an AC recording at high gain; (Middle) trace of a DC recording at low gain; (Lower), the number of spontaneous and serotonin-evoked muscle responses per sec. (B) [<sup>3</sup>H]Acetylcholine ([<sup>3</sup>H]ACh) release from hybrid cells into the medium. Cells were grown in the presence of 1 mM Bt<sub>2</sub>cAMP for 17 days and then transferred to capillary pipettes (21) and grown for an additional 2 days. Cells then were incubated in 25  $\mu$ M [<sup>3</sup>H]methylcholine (4.2 Ci/mmol) for 45 min and washed by perfusion (0.4 ml/min) for 17.5 min before 1-ml fractions were collected. [<sup>3</sup>H]Acetylcholine in the perfusate was separated from [<sup>3</sup>H]methylcholine and measured (21). Cells were exposed to 10  $\mu$ M serotonin (5HT) with 50 nM ( $\square$ ), 500 nM ( $\Delta$ ), or no ( $\circ$ ) LSD at the times shown.

or mimicked by LSD. Serotonin also stimulates adenylate cyclase activity of NCB-20 hybrid cells, and in contrast these receptors do not desensitize. In addition, LSD activates adenylate cyclase and partially inhibits activation of the enzyme by serotonin. The results suggest that cell depolarization and activation of adenylate cyclase are mediated by different species of serotonin receptors.

The serotonin receptors that mediate excitatory, depolarizing responses resemble M-receptors of neurons in the peripheral nervous system (6). These receptors differ from both pre- and postsynaptic serotonin receptors of mammalian brainstem (5, 7), which inhibit neuronal firing and are also activated by LSD. Inhibitory serotonin responses not affected by LSD have been found in brainstem neurons (22), the suprachiasmatic nucleus (4), and half the serotonin-sensitive neurons of cat cerebral cortex (23). The specificity of serotonin receptors coupled to activation of adenylate cyclase in NCB-20 hybrid cells resembles that of serotonin receptors coupled to adenylate cyclase in mammalian brain (3).

The demonstration that activation of adenylate cyclase by serotonin is stimulated by GTP, that sodium fluoride uncouples activation by serotonin, and that serotonin inhibits to a small extent the stimulation of adenylate cyclase by  $PGE_1$  suggests a common mechanism for the coupling of receptors for serotonin and other neurotransmitters to the adenylate cyclase complex. However, the additive, rather than synergistic effects of serotonin and  $Gpp(NH)p$  suggest that coupling of the serotonin receptors to the adenylate cyclase complex may differ from that of other neurotransmitters. Serotonin stimulates adenylate cyclase activity 50–100% in homogenates of colliculus of neonatal rat brain, which is similar to the extent of activation of adenylate cyclase by serotonin in NCB-20 homogenates.

Eadie-Scatchard analysis of the activation of adenylate cyclase by serotonin suggests a bimolecular interaction and reveals no evidence of receptor heterogeneity. The Hill interaction coefficient ( $n$ ) is 1.0, indicating independent, noncooperative reactions. LSD activates adenylate cyclase ( $K_{act} = 12$  nM) and inhibits the activation of the enzyme by serotonin ( $K_i = 10$  nM). In addition, mianserin and cyproheptadine inhibit serotonin activation of adenylate cyclase ( $K_i = 43$  nM and 95 nM, respectively) and LSD activation of adenylate cyclase ( $K_i = 100$  nM and 64 nM, respectively). These results show that serotonin and LSD interact at a receptor site(s) that mediates activation of adenylate cyclase. Enjalbert *et al.* (3) have shown a complex interaction between serotonin and LSD at the level of adenylate cyclase in mammalian brain. Interactions between serotonin and LSD have also been demonstrated in binding studies (24, 25).

Binding sites for  $[^3H]$ LSD were detected in NCB20 homogenates (unpublished results); the  $K_{D_{app}}$  was 36 nM, the Hill coefficient was 1.0, and the receptor concentration was 385 fmol/mg of protein.  $[^3H]$ LSD was displaced by serotonin ( $K_i = 110$ –180 nM). These results agree well with those presented for the stimulation of adenylate cyclase activity by a serotonin receptor that is also responsive to LSD. Two binding sites for  $[^3H]$ serotonin were detected in NCB-20 homogenates [ $K_{D_{app}} = 200$  nM and 3750 nM] and serotonin and LSD interactions

also were shown. LSD does not displace serotonin from the low-affinity serotonin site, which suggests that these molecules may function as receptors mediating cell depolarization and acetylcholine release.

We conclude that NCB-20 hybrid cells possess two species of serotonin receptors, that activation of adenylate cyclase does not affect the rate of acetylcholine release, and, conversely, that serotonin-dependent cell depolarization does not affect intracellular levels of cAMP or cGMP in the hybrid cells tested.

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